(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: DIAGNOSTIC AND THERAPEUTIC USE OF STEROIDOGENIC ACUTE REGULATORY PROTEIN FOR NEURODEGENERATIVE DISEASES

**Identification of genes involved in
Alzheimer's Disease pathology**

(57) Abstract: The present invention discloses the differential expression of the steroidogenic acute regulatory protein (StAR) gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or for determining whether a subject is at increased risk of developing such a disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using the StAR gene and gene products. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

WO 03/104811 A2

DIAGNOSTIC AND THERAPEUTIC USE OF STEROIDOGENIC ACUTE REGULATORY PROTEIN FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. AD is the most common neurodegenerative disease, accounting for about 70% of all dementia cases, and it is probably the most devastating age-related neurodegenerative condition affecting about 10% of the population over 65 years of age and up to 45% over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles.

The amyloid-β (Aβ) protein evolves from the cleavage of the amyloid precursor protein (APP) by different kinds of proteases. The cleavage by the β/γ-secretase leads to the formation of Aβ peptides of different lengths, typically a short more soluble and slow aggregating peptide consisting of 40 amino acids and a longer 42 amino acid peptide, which rapidly aggregates outside the cells, forming the characteristic amyloid plaques (Selkoe, *Physiological Rev* 2001, 81: 741-66; Greenfield et al., *Frontiers Bioscience* 2000, 5: D72-83). Two types of plaques, diffuse plaques and neuritic plaques, can be detected in the brain of AD patients, the latter ones being the classical, most prevalent type. They are primarily found in the cerebral cortex and hippocampus. The neuritic plaques have a diameter of 50μm to 200μm and are composed of insoluble fibrillar amyloids, fragments of dead

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neurons, of microglia and astrocytes, and other components such as neurotransmitters, apolipoprotein E, glycosaminoglycans, α1-antichymotrypsin and others. The generation of toxic Aβ deposits in the brain starts very early in the course of AD, and it is discussed to be a key player for the subsequent destructive processes leading to AD pathology. The other pathological hallmarks of AD are neurofibrillary tangles (NFTs) and abnormal neurites, described as neuropil threads (Braak and Braak, *Acta Neuropathol* 1991, 82: 239-259). NFTs emerge inside neurons and consist of chemically altered tau, which forms paired helical filaments twisted around each other. Along the formation of NFTs, a loss of neurons can be observed. It is discussed that said neuron loss may be due to a damaged microtubule-associated transport system (Johnson and Jenkins, *J Alzheimers Dis* 1996, 1: 38-58; Johnson and Hartigan, *J Alzheimers Dis* 1999, 1: 329-351). The appearance of neurofibrillary tangles and their increasing number correlates well with the clinical severity of AD (Schmitt et al., *Neurology* 2000, 55: 370-376).

AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. The cognitive disturbances include among other things memory impairment, aphasia, agnosia and the loss of executive functioning. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-92).

The age of onset of AD may vary within a range of 50 years, with early-onset AD occurring in people younger than 65 years of age, and late-onset of AD occurring in those older than 65 years. About 10% of all AD cases suffer from early-onset AD, with only 1-2% being familial, inherited cases.

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon 4 allele of the three different existing alleles (epsilon 2, 3, and 4) of the apolipoprotein E gene (ApoE) (Strittmatter et al., *Proc Natl Acad Sci USA* 1993, 90: 1977-81; Roses, *Ann NY Acad Sci* 1998, 855: 738-43). Efforts to detect further susceptibility genes and disease-linked

polymorphisms lead to the assumption that specific regions and genes on human chromosomes 10 and 12 may be associated with late-onset AD (Myers et al., *Science* 2000, 290: 2304-5; Bertram et al., *Science* 2000, 290: 2303; Scott et al., *Am J Hum Genet* 2000, 66: 922-32).

Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP) on chromosome 21, presenilin-1 on chromosome 14, and presenilin-2 on chromosome 1, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The mutations found to date account for only half of the familial AD cases, which is less than 2% of all AD patients. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, agents, compositions, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

The brain is a target site of peripheral steroid hormones synthesized and secreted into circulation by the adrenal glands, gonads and the feto-placental axis. It is well-known, for instance, that gonadal androgens act on the brain to influence the reproductive behaviour of individuals. However, the brain is also a site of synthesis of steroid hormones, a process termed neurosteroidogenesis. Only two decades have passed from the first description of dehydroepiandrosterone sulfate production in rodent brain (Corpechot et al., *Proc. Natl. Acad. Sci. U.S.A.* 1981, 78:4704-4707) and, therefore, this field of research is as a young discipline at the interface of neurology, endocrinology, and behavioral sciences.

There are two principle categories of steroid hormone action in brain tissue, a genomic action and an immediate non-genomic action. At the genomic level, steroid hormones bind to intracellular/nuclear hormone receptors that regulate transcription of target genes. These adaptive responses generally occur within hours or days. In addition, steroid hormones show an immediate impact on the excitability of the central nervous system through action on neurotransmitter-gated ion channels (for

review: Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679). In particular, pregnenolone sulfate potentiates the calcium conductivity of N-methyl-D-aspartate receptors and suppresses the chloride conductivity of the gamma-aminobutyric acid receptors in rat hippocampal neurons, thus affecting the overall neuronal excitability (Irwin et al., *Neurosci. Lett.* 1992, 141:30-34; Harrison et al., *J. Neurosci.* 1987, 7: 604-609).

In order to understand the physiological roles of neurosteroidogenesis in the mammalian brain it is important to identify the brain regions involved and the type(s) of cells that are proficient for the key enzymes and therefore likely to be active in the synthetic process. Most of the current knowledge in this regard is derived from studies in rodents, and the overall picture that emerges is that glial cells, oligodendrocytes and astrocytes play a major role in neurosteroid formation and metabolism in cerebrum and cerebellum (for review: Tsutsui et al., *Neurosci. Res.* 2000, 36:261-273). In more recent studies in the rat system, on the other hand, pyramidal and granule neurons of the hippocampus (Furukawa et al., J. *Neurochem.* 1998, 71:2231-2238; Kimoto et al., *Endocrinology* 2001, 142:3578-3589) and Purkinje cell neurons of the cerebellum (Ukena et al., *Endocrinology* 1998, 139:137-147; Ukena et al., *Endocrinology* 1999, 140:805-813) were shown to possess the key enzymes of neurosteroidogenesis as well. Due to the difficulty of obtaining fresh brain material, analogous studies in the human system are in their infancy. Using a sensitive reverse transcriptase polymerase chain reaction approach, the messenger RNA for the rate-limiting enzyme of neurosteroidogenesis, CYP11A1; was detected in surgical specimen of human brain cortices of the frontal and temporal lobes and the hippocampus, albeit at levels 200 times lower than in the adrenal gland (Watzka et al., *J. Neuroendocrinol.* 1999,11: 901-905). Messenger RNAs for two other key enzymes of neurosteroidogenesis, CYP21 and 17-beta hydroxysteroid dehydrogenase, were also detected in surgical specimen of the temporal lobe and hippocampus (Beyenburg et al., *Neurosci. Lett.* 2001, 308:111-114; Stoffel-Wagner et al., *J. Endocrinol.* 1999, 160:119-126). Based on this small complement of enzymes, seven different steroids can principally be synthesized in situ, including pregnenolone and progesterone. However, additional neurosteroidogenic enzymes have been detected in brain, including CYP19 (aromatase), 5-alpha-reductase, 3-alpha-hydroxysteroid dehydrogenase, CYP17, CYP11B1 and others (for review: Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679) and, therefore, many more neurosteroids are likely synthesized within the brain.

To a large extent the potential physiological and pathophysiological implications of the above findings remain to be elucidated. For instance, the anticonvulsive, anesthetic, and anxiolytic effects of neuroactive steroids are attributed to their capacity to modulate the gamma-aminobutyric acid receptor function and are therefore of immediate, non-genomic nature (Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679). Furthermore, it has been postulated that neurosteroids act on nerve cells through membrane receptors coupled to G proteins, various neuropeptide receptors and progesterone receptors (Orchinik et al., *Proc. Natl. Acad. Sci. U.S.A.* 1992, 89:3830-3834; Grazzini et al., *Nature* 1998, 392:509-512; Rupprecht et al., *Neuron* 1993, 11:523-530). On a more phenomenological level, the abundant neurosteroids dehydroepiandrosterone and dehydroepiandrosterone sulfate, generated from pregnenolone by action of cytochrome P450 17-alpha-lyase and hydroxysteroid sulfotransferase, are discussed as neuroprotective agents, whose levels decrease with age and under stress (Kimonides et al., *Proc. Natl. Acad. Sci. U.S.A.* 1998, 95:1852-1857). A potential role of pregnenolone, dehydroepiandrosterone and their sulfate esters in learning, memory and cognitive aging is under controversial discussion (Vallee et al., *Brain Research Reviews* 2001, 37:301-312; WO 01/55692). Fluctuations in neuroactive steroids are thought to contribute to the increased risk of developing psychiatric diseases in women during the perimenstrual phase, pregnancy, the post partum period, and menopause. On the cognitive level, estrogens and androgens impact on verbal fluency, the performance of spatial tasks, verbal memory tests, and fine-motor skills (for review: McEwen, *Annu. Rev. Neurosci.* 1999, 22:105-122; Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679).

The instant invention discloses the differential expression of the steroidogenic acute regulatory protein (StAR) gene in different regions of the AD brain but not in the brain of healthy age-matched control individuals. StAR is encoded by the synonymous gene on human chromosome 8p11.2 (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92:4778-4782; GenBank accession number U17280; US patent 6194555; US patent 5807678). StAR is a 285 amino acids protein partly located in mitochondria and synthesized as a conformationally labile precursor with an N-terminal mitochondrial targeting sequence on cytoplasmic ribosomes (Stocco, *Biochim. Biophys. Acta* 2000, 1486:184-197, GenBank accession number P49675). By virtue of its so called START domain, StAR stoichiometrically binds cholesterol within the cytoplasm and shuttles the hydrophobic cargo through both mitochondrial

membranes and the enclosed intermembrane space. Within the inner mitochondrial membrane cholesterol is transferred to CYP11A1 which then catalyzes the conversion of cholesterol to pregnenolone as the committed step of neurosteroidogenesis (Stocco, *Biochim. Biophys. Acta* 2000, 1486: 184-197). Multiple transcripts of StAR were found in human steroidogenic tissues, in particular ovary, testis, adrenal gland, and within the kidney (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92:4778-4782). However, the same study did not identify StAR expression in human brain. Expression of StAR is under control of the acute regulation by tropic hormones that generally act via the cAMP second messenger system. In case of the adrenal and gonads these tropic hormones are pituitary gland derived corticotropin and luteinizing hormone, while in the kidney, expression of StAR responds to parathyroid hormone (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92:4778-4782). In addition to regulation of gene expression, StAR protein activity or stability is thought to be regulated by cAMP dependent protein kinase / protein kinase A (Clark et al., *Endocrine Res.* 2000, 26:681-689).

Mutations in the StAR gene have been associated with severe human disease conditions termed lipoid congenital adrenal hyperplasia and dose sensitive sex reversal (Lin et al., *Science* 1995, 267:1828-1831; Tee et al., *Hum. Molec. Genet.* 1995, 4:2299-2305; US patent 6194555, US patent 5872230, US patent 5807678, WO 9629338). More recently, overexpression and/or amplification of the StAR gene was discovered in a number of types of cancer cells, in particular breast cancer cells (WO 131342). To date, no mutations in the StAR gene have been described to be associated with neurodegenerative diseases. Likewise, no experiments have been described that demonstrate a relationship between the dysregulation of the StAR gene and the pathology of neurodegenerative diseases, in particular Alzheimer's disease. The disclosures of the instant invention offer new ways, inter alia, for the diagnosis and treatment of said diseases.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level,

or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. "Dysregulation" shall mean an upregulation or downregulation of gene expression. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "ORF" is an acronym for "open reading frame" and refers to a nucleic acid sequence that does not possess a stop codon in at least one reading frame and therefore can potentially be translated into a sequence of amino acids. "Regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological,

functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids, natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or anorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or "primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may preferably vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. As used herein, "homolog or homology" is a term used in the art to describe the relatedness of a nucleotide or peptide sequence to another nucleotide or peptide sequence, which is determined by the degree of identity and/or similarity between said sequences compared. The term "variant" as used herein refers to any polypeptide or protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention. Furthermore, the term "variant" shall include any shorter or longer version of a polypeptide or protein. "Variants" shall also comprise sequences that have at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of the human StAR gene, SEQ ID NO. 1. "Variants" of a protein molecule include, for example, proteins with conservative amino acid substitutions in highly conservative regions. "Proteins and polypeptides" of the present invention

include variants, fragments and chemical derivatives of the protein comprising the amino acid sequence of StAR, SEQ ID NO. 1. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants. The term "isolated" as used herein is considered to refer to molecules that are removed from their natural environment, i.e. isolated from a cell or from a living organism in which they normally occur, and that are separated or essentially purified from the coexisting components with which they are found to be associated in nature. This notion further means that the sequences encoding such molecules can be linked by the hand of man to polynucleotides, to which they are not linked in their natural state, and that such molecules can be produced by recombinant and/or synthetic means. Even if for said purposes those sequences may be introduced into living or non-living organisms by methods known to those skilled in the art, and even if those sequences are still present in said organisms, they are still considered to be isolated. In the present invention, the terms "risk", "susceptibility", and "predisposition" are tantamount and are used with respect to the probability of developing a neurodegenerative disease, preferably Alzheimer's disease.

The term 'AD' shall mean Alzheimer's disease. "AD-type neuropathology" as used herein refers to neuropathological, neurophysiological, histopathological and clinical hallmarks as described in the instant invention and as commonly known from state-of-the-art literature (see: Iqbal, Swaab, Winblad and Wisniewski, *Alzheimer's Disease and Related Disorders (Etiology, Pathogenesis and Therapeutics)*, Wiley & Sons, New York, Weinheim, Toronto, 1999; Scinto and Daffner, *Early Diagnosis of Alzheimer's Disease*, Humana Press, Totowa, New Jersey, 2000; Mayeux and Christen, *Epidemiology of Alzheimer's Disease: From Gene to Prevention*, Springer Press, Berlin, Heidelberg, New York, 1999; Younkin, Tanzi and Christen, *Presenilins and Alzheimer's Disease*, Springer Press, Berlin, Heidelberg, New York, 1998).

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, argyrophilic grain dementia and other tauopathies, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are,

for instance, age-related macular degeneration, narcolepsy, motor neuron diseases, prion diseases, traumatic nerve injury and repair, and multiple sclerosis.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences of the StAR gene, or fragments, or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects. Whether an amplification results in a specific nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimer's disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said gene coding for a human phosphoprotein, is the gene coding for the human steroidogenic acute regulatory protein (StAR), also termed start domain-containing protein 1 (STARD1), or cycloheximide sensitive factor, represented by SEQ ID NO. 1 (GenBank accession number P49675; mRNA GenBank accession number U17280).

In a further preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said neurodegenerative disease or disorder is Alzheimer's disease, and said subjects suffer from Alzheimer's disease.

The present invention discloses the differential expression and regulation of the StAR gene in specific brain regions of AD patients. Consequently, the StAR gene and its corresponding transcription and/or translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively, StAR transcription and/or translation products may confer a

neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is selected from the group comprising brain tissue, or other tissues, organs, or body cells. The sample can also comprise cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or mucus. Preferably, the methods of diagnosis, prognosis, monitoring the progression or evaluating a treatment for a neurodegenerative disease, according to the instant invention, can be practiced *ex corpore*, and such methods preferably relate to samples, for instance, body fluids or cells, removed, collected, or isolated from a subject or patient.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an alteration in the level and/or activity of a transcription product of the StAR gene and/or of a translation product of the StAR gene and/or of a fragment, or derivative, or variant of said transcription or translation product, in a sample cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neuro-degenerative disease, particularly AD.

In preferred embodiments, the measurement of a level of a transcription product of the StAR gene is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based microarray technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000). An example of an immunoassay is the detection and measurement of enzyme activity as disclosed and described in the patent application WO 02/14543.

Furthermore, a level and/or an activity of a translation product of the StAR gene and/or a fragment, or variant, or derivative of said translation product, and/or the level of activity of said translation product, and/or of a fragment, or variant, or derivative thereof, can be detected using an immunoassay, an activity assay, and/or a binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R, *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford; England, 1999). Enzymatic activity of a translation product of the StAR gene, may be measured by *in vitro*, cell-based, or *in vivo* assays. All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000).

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the

propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the StAR gene (ii) reagents that selectively detect a translation product of the StAR gene; and

(b) an instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the StAR gene, in a sample from said subject; and

- diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD, in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the StAR gene, and/or (ii) a transcription

product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation .product of the StAR gene, or a fragment, or derivative, or a variant thereof. An agent for treating or preventing a neurodegenerative disease, in particular AD, according to the instant invention, may also consist of a nucleotide, an oligonucleotide, or a polynucleotide. Said oligonucleotide or polynucleotide may comprise a nucleotide sequence of the gene coding for StAR, either in sense orientation or in antisense orientation.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease. has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred

embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcription products of the StAR gene. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way: Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy. A recently developed method of regulating the intracellular expression of genes by the use of double-stranded RNA, known variously as RNA interference (RNAi), can be another effective approach for nucleic acid therapy (Hannon, *Nature* 2002, 418: 244-251).

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection or liposomal mediated transfection (see Mc Celland and Pardee, *Expression- Genetics: Accelerated and High-Throughput Methods*, Eaton Publishing, Natick, MA, 1999).

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier

refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for StAR, or a fragment, or a derivative, or a variant thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar

to a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York and Jackson and Abbott, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England, 1999). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease. Such an animal may be useful for screening, testing and validating compounds, agents and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment, or derivative, or variant of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level

of said substances in a matched control animal which is equally predisposed to developing or has already developed said symptoms of said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances-in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses the StAR gene, or a fragment, or derivative, or variant thereof, under the control of a transcriptional regulatory element which is not the native StAR gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and a translation product of the StAR gene, or a fragment, or derivative, or variant thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding a detectable, preferably a fluorescently labelled ligand to said containers, and (iv) incubating said translation product of the StAR gene, or said fragment, or derivative, or variant thereof, and said compound or plurality of compounds, and said detectable, preferably fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said translation product of the StAR gene, or with said fragment, or derivative, or variant thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said translation product of the StAR gene, or said fragment, or derivative, or variant thereof. It might be preferred to reconstitute said StAR translation product, or fragment, or derivative, or variant thereof into artificial

liposomes to generate the corresponding proteoliposomes to determine the inhibition of binding between a ligand and said StAR translation product. Methods of reconstitution of StAR translation products from detergent into liposomes have been detailed (Schwarz et al., *Biochemistry* 1999, 38: 9456-9464; Krivosheev and Usanov, *Biochemistry-Moscow* 1997, 62: 1064-1073). Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a translation product of the StAR gene, or a fragment, or derivative, or variant thereof. One example of a fluorescent binding assay, in this case based on the use of carrier particles, is disclosed and described in patent application WO 00/52451. A further example is the competitive assay method as described in patent WO 02/01226. Preferred signal detection methods for screening assays of the instant invention are described in the following patent applications: WO 96/13744, WO 98/16814, WO 98/23942, WO 99/17086, WO 99/34195, WO 00/66985, WO 01/59436, WO 01/59416.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a translation product of the StAR gene by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a translation product of the StAR gene, or to a fragment, or derivative, or variant thereof. Said screening assay comprises (i) adding a liquid suspension of a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a detectable, preferably a fluorescently labelled compound or a plurality of detectable, preferably fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said translation product of the StAR gene, or said fragment, or derivative, or variant thereof, and said detectable, preferably fluorescently labelled compound or detectable, preferably fluorescently labelled

compounds, and (iv) measuring the amounts of preferably the fluorescence associated with said translation product of the StAR gene, or with said fragment, or derivative, or variant thereof, and (v) determining the degree of binding by one or more of said compounds to said translation product of the StAR gene, or said fragment, or derivative, or variant thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said assay methods may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a translation product of the StAR gene, or a fragment, or derivative, or variant thereof.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a StAR gene product by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

The present invention features a protein molecule shown in SEQ ID NO. 1, said protein molecule being a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

The present invention also features a protein molecule shown in SEQ ID NO. 1, said protein molecule being a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the StAR gene, or a fragment, or derivative, or variant thereof. The immunogen may comprise

immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof (see Dubel and Breitling, *Recombinant Antibodies*, Wiley-Liss, New York, NY, 1999). Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods, based on state-in-the-art techniques (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R., *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford, England, 1999) such as enzyme-immuno assays (e.g. enzyme-linked immunosorbent assay, ELISA), radioimmuno assays, chemoluminescence-immuno assays, Western-blot, immunoprecipitation and antibody microarrays. These methods involve the detection of translation products of the StAR gene, or fragments, or derivatives, or variants thereof.

In a preferred embodiment of the present invention, said antibodies can be used for detecting a pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, a pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173.

Other features and advantages of the invention will be apparent from the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., Annals of Neurology 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F), the temporal cortex (T), and the hippocampus (H) of AD patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figures 2 and 3 demonstrate the differential expression of the StAR gene in AD brain tissues by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and the temporal cortex (T) of AD patients (Figure 2a) and samples from the frontal cortex (F) and the hippocampus (H) of AD patients (Figure 3a) was performed by the LightCycler rapid thermal cycling technique. Likewise, samples of healthy, age-matched control individuals were compared (Figure 2b for frontal cortex and temporal cortex, Figure 3b for frontal cortex and hippocampus). The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for cyclophilin B, the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figures depict the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of the StAR cDNAs from both, the frontal and temporal cortices of a normal control individual, and from the frontal cortex and hippocampus of a normal control individual, respectively, during the exponential phase of the reaction are juxtaposed (Figures 2b and 3b, arrowheads), whereas in Alzheimer's disease (Figures 2a and 3a, arrowheads) there is a significant separation of the

corresponding curves, indicating a differential expression of the StAR gene in the respective analyzed brain regions.

Figure 4 charts the schematic alignment of StAR primer sequences to the nucleotide sequence of the human StAR gene (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92: 4778-4782; GenBank accession number U17280); polyA: polyadenylation site; primer positions P1, P2 are indicated by arrows; the open box represents the StAR open reading frame (positions 127-984).

Figure 5 shows the exact sequence alignments of the StAR primer pair to the nucleotide sequence of the human StAR gene (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92: 4778-4782; GenBank accession number U17280).

Figure 6 discloses SEQ ID NO. 1, the polypeptide sequence of human StAR, comprising 285 amino acids (GenBank accession number P49675).

Figure 7 depicts human cerebral cortex labeled with anti-StAR rabbit polyclonal antibodies (green signals). Immunoreactivity of StAR was detected in both the pre-central cortex (CT) and in the white matter (WM) (Figure 7a, low magnification). In the cortex, StAR immunoreactivity appears as punctate spots in neuronal and glial cytoplasma and in neurites, which indicates a mitochondrial localization (Figure 7b, high magnification). In the white matter, punctate signals were detected in the cytoplasma of many glial cells. Blue signals indicate nuclei stained with DAPI.

Table 1 lists the gene expression levels in the frontal cortex relative to the temporal cortex for the StAR gene in seven Alzheimer's disease patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P017, P019 (1.72 to 7.30 fold) and five healthy, age-matched control individuals, herein identified by internal reference numbers C005, C008, C011, C012, C014 (0.59 to 2.25 fold). The scatter plot diagram visualizes individual values of the frontal to temporal cortex regulation ratios in control samples (dots) and in AD patient samples (triangles), respectively. The values shown are reciprocal values according to the formula described herein (see below).

Table 2 lists StAR gene expression levels in the frontal cortex relative to the hippocampus in six Alzheimer's disease patients, herein identified by internal

reference numbers P010, P011, P012, P014, P016, P019 (2.24 to 25.61 fold) and two healthy, age-matched control individuals, herein identified by internal reference numbers C005, C008 (2.65 and 3.56 fold). The scatter plot diagram visualizes individual values of the frontal cortex to hippocampus regulation ratios in control samples (dots) and in AD patient samples (triangles), respectively. The values shown are reciprocal values according to the formula described herein (see below).

EXAMPLE I:

(i)   Brain tissue dissection from patients with AD:
Brain tissues from AD patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Figure 1) and stored at −80 °C until RNA extractions were performed.

(ii)   Isolation of total mRNA:
Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were used to generate a melting curve with the LightCycler technology as described in the manufacturer's protocol (Roche).

(iii)   Quantitative RT-PCR analysis:
The expression levels of the human StAR gene in temporal versus frontal cortex and in frontal cortex versus hippocampus were analyzed using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint readout. The ratios of StAR cDNA from the frontal cortex and temporal cortex, and from the frontal cortex and hippocampus, respectively, were determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for human StAR (5'-CCAATGTCAAGGAGATCAAGGTC-3' and 5'-GCCAGCTCGTGAGTAATGAATGT-3'). PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 80°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 67 bp for StAR was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTTTGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'.

Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for StAR and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex, and from frontal cortex and hippocampus, respectively, were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \char`\^ (\, (C_t \text{ value - intercept}) / \text{slope}) \quad [\text{ng total brain cDNA}]$$

The values for frontal and temporal cortex cDNAs of StAR, and the values for frontal cortex and hippocampus StAR cDNAs, respectively, were normalized to cyclophilin B and the ratios were calculated according to formulas:

$$\text{Ratio} = \frac{\text{StAR temporal [ng] / cyclophilin B temporal [ng]}}{\text{StAR frontal [ng] / cyclophilin B frontal [ng]}}$$

$$\text{Ratio} = \frac{\text{StAR hippocampus [ng] / cyclophilin B hippocampus [ng]}}{\text{StAR frontal [ng] / cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios, and of the hippocampal to frontal ratios, respectively, of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for StAR to the mean average value of the set of reference standard genes instead of normalizing to one single

gene alone. The calculation was performed by dividing the respective ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of such quantitative RT-PCR analysis for the StAR gene are shown in Figures 2 and 3.

(iv)   Immunohistochemistry:

For immunofluorescence staining of StAR in human brain, frozen sections were prepared with a cryostat (Leica CM3050S) from post-mortem pre-central gyrus of a donor person and fixed in acetone for 10 min. After washing in PBS, the sections were pre-incubated with blocking buffer (10% normal goat serum, 0.2% Triton X-100 in PBS) for 30 min and then incubated with affinity-purified anti-StAR rabbit polyclonal antibodies (1:50 diluted in blocking buffer; Dianova, Hamburg) overnight at 4°C. After rinsing three times in 0.1% Triton X-100/PBS, the sections were incubated with FITC-conjugated goat anti-rabbit IgG (1:150 diluted in 1% BSA/PBS) for 2 hours at room temperature and then again washed in PBS. Staining of the nuclei was performed by incubation of the sections with 5µM DAPI in PBS for 3 min (blue signal). In order to block the autofluoresence of lipofuscin in human brain, the sections were treated with 1% Sudan Black B in 70% ethanol for 2-10 min at room temperature and then sequentially dipped in 70% ethanol, destilled water and PBS. The sections were coverslipped with 'Vectrashield' mounting medium (Vector Laboratories, Burlingame, CA) and observed under an inverted microscope (IX81, Olympus Optical). The digital images were captured with the appropriate software (AnalySiS, Olympus Optical).

CLAIMS

1.   A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising determining a level and/or an activity of

(i)   a transcription product of the StAR gene, and/or .

(ii)·   a translation product of the StAR gene, and/or

(iii)   a fragment, or derivative, or· variant of said transcription or translation product,

in a sample ·from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said ·neurodegenerative disease...

2. The method according to claim 1 wherein said neurodegenerative disease is Alzheimer's disease.

3. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of. a subject to develop such a disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the StAR gene and (ii) reagents that selectively detect a translation product of the StAR gene and

(b) an instruction for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the StAR gene, in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value

representing a known disease status indicates a diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

4. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

(i)   the StAR gene, and/or

(ii)   a transcription product of the StAR gene, and/or

(iii) a translation product of the StAR gene, and/or

(iv)   a fragment, or derivative, or variant of (i) to (iii).

5. A recombinant, non-human animal comprising a non-native gene sequence coding for StAR, or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

(i)   providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii)   introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv)   transplanting said embryo into a pseudopregnant non-human animal, and

(v)   allowing said embryo to develop to term, and

(vi)   identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

6. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i)   the StAR gene, and/or

(ii)   a transcription product of the StAR gene, and/or

(iii) a translation product of the StAR gene, and/or

(iv)   a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or-more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

7. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the StAR gene, and/or

(ii) a transcription product of the StAR gene, and/or

(iii) a translation product of the StAR gene, and/or

(v) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;

(d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

8. The method according to claim 7 wherein said test animal and/or said control animal is a recombinant animal which expresses StAR, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional control element which is not the native StAR gene transcriptional control element.

9. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and a translation product of the StAR gene, or a fragment, or a derivative, or a variant thereof, said assay comprising the steps of:

(i)   adding a liquid suspension of said translation product of the StAR gene, or a fragment, or derivative, or variant thereof, to a plurality of containers;

(ii)   adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers;

(iii)   adding a detectable, preferably a fluorescently labelled ligand to said containers;

(iv)   incubating said translation product of the StAR gene, or said fragment, or derivative, or variant thereof, and said compound or compounds, and said detectable, preferably fluorescently labelled ligand;

(v)   measuring amounts of preferably fluorescence associated with said translation product of the StAR gene, or with said fragment, or derivative, or variant thereof; and

(vi)   determining the degree of inhibition by one or more of said compounds of binding of said ligand to said translation product of the StAR gene, or said fragment, or derivative, or variant thereof.

10. A protein molecule, said protein molecule being a translation product of the StAR gene, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

11. A protein molecule, said protein molecule being a translation product of the StAR gene, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

12. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the StAR gene, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for detecting a pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell.

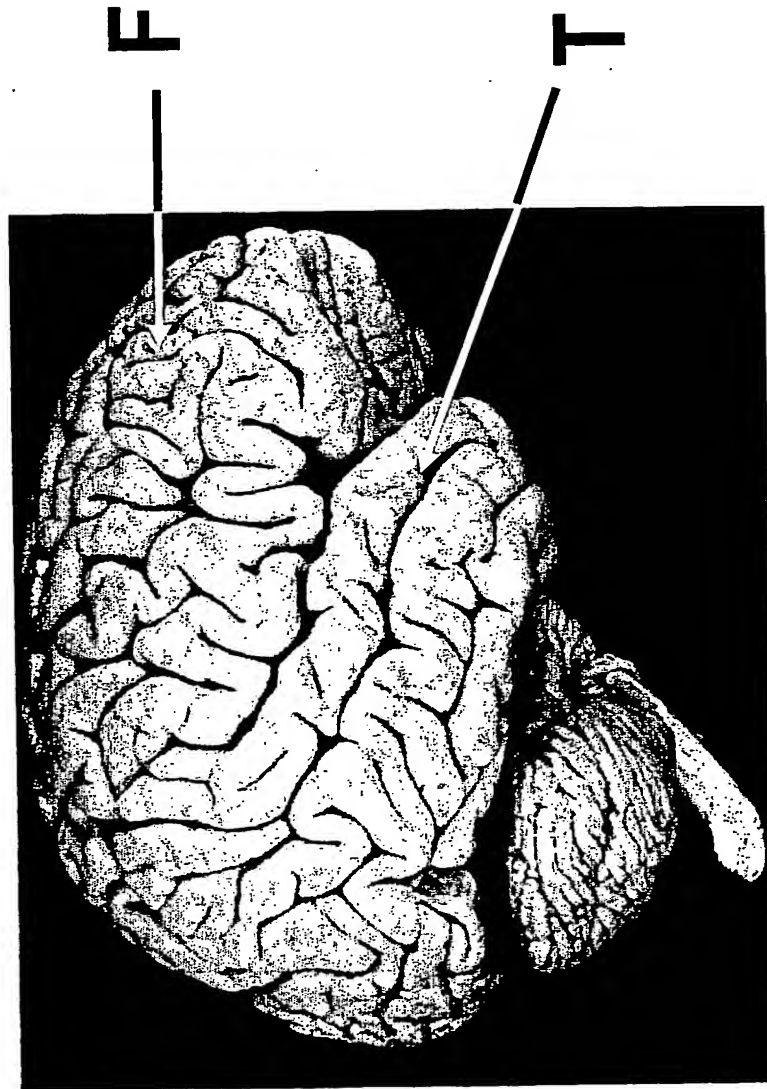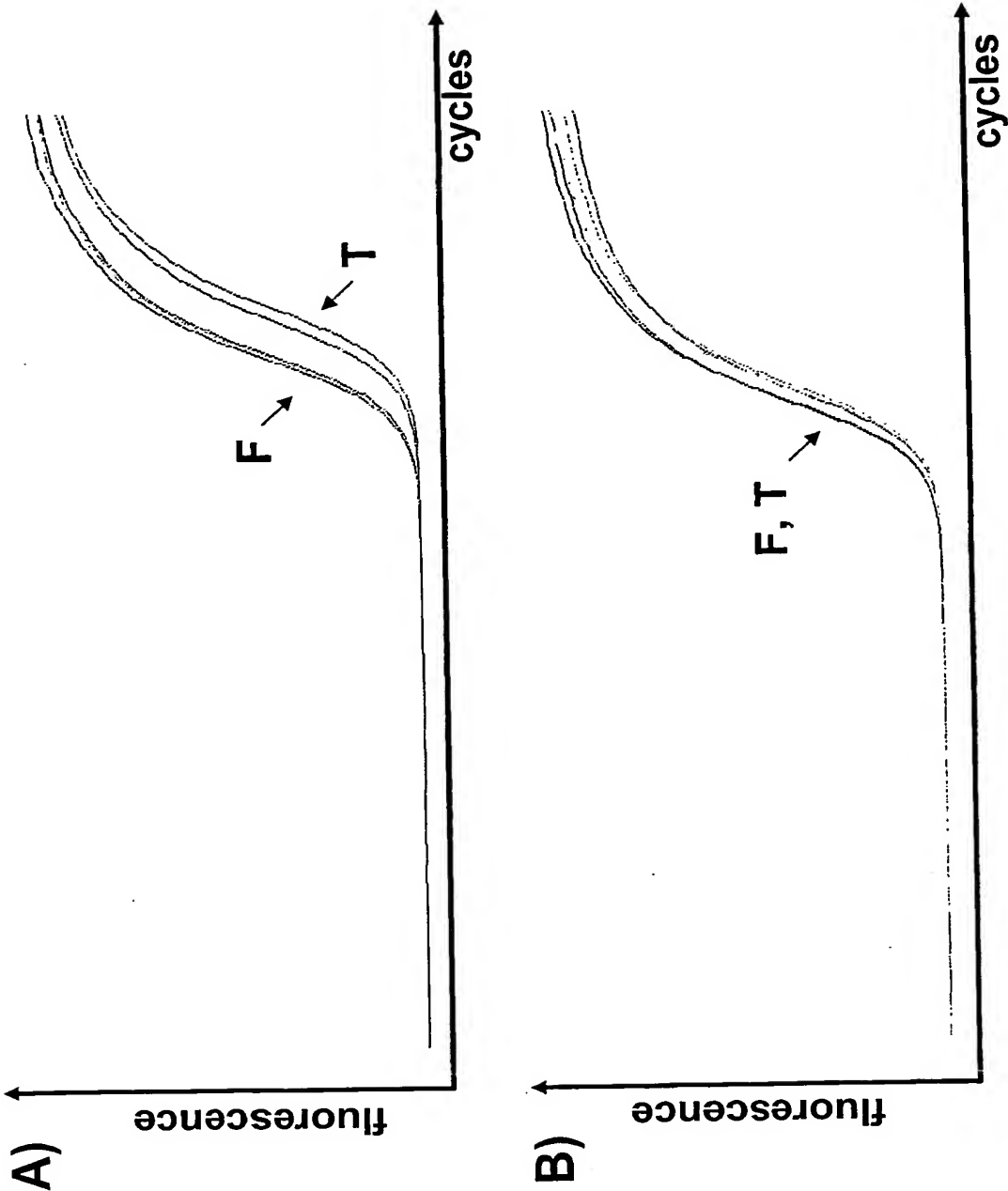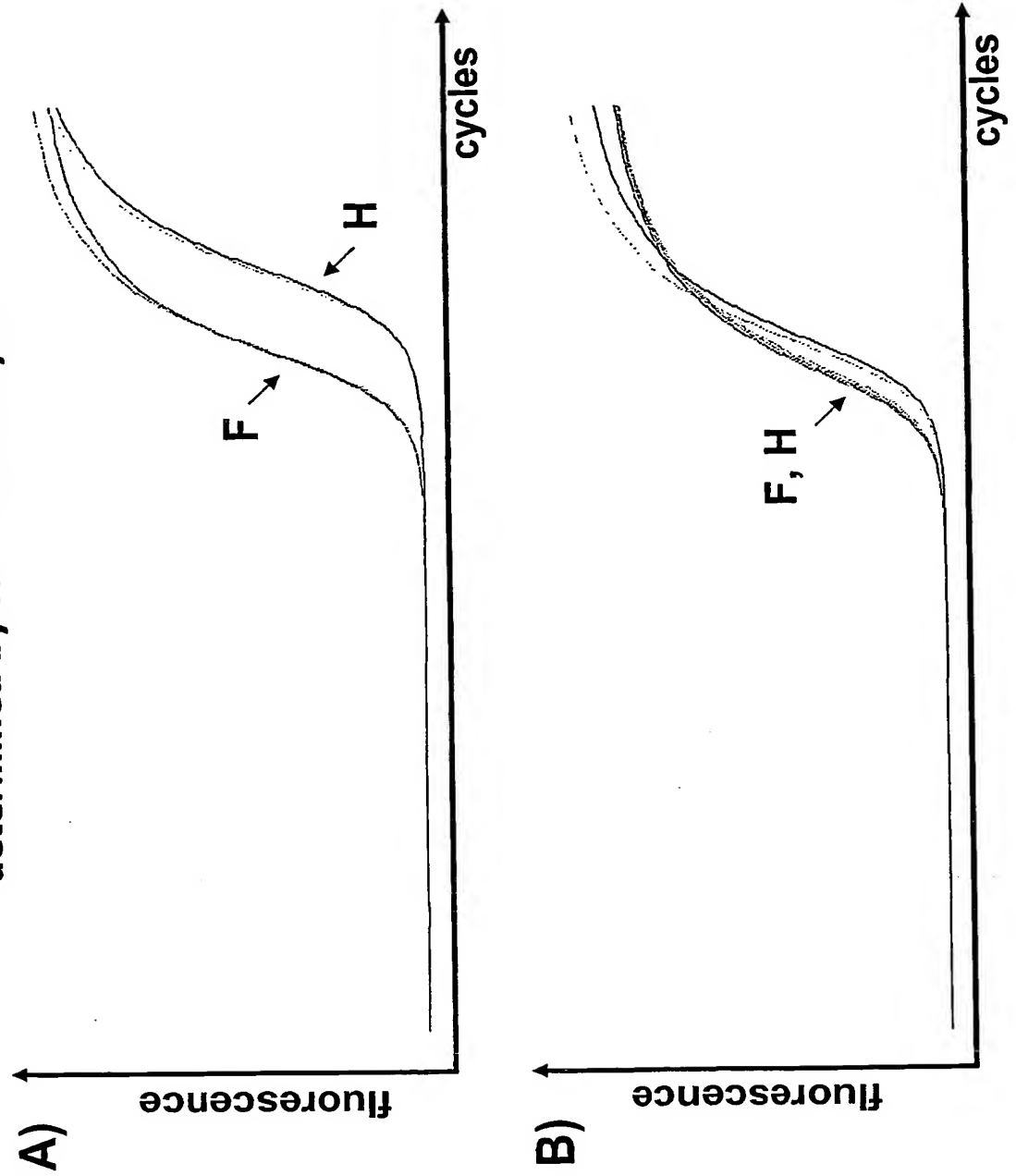Fig. 1: Identification of genes involved in Alzheimer's Disease pathology

Fig. 2: Differential expression of StAR as determined by RT- PCR analysis

Fig. 3: Differential expression of StAR as
determined by RT- PCR analysis

Fig. 4: Schematic alignment of StAR RT-PCR primers with human StAR cDNA (GenBank accession number U17280)

-5/9-

Fig. 5: Alignment of StAR RT-PCR primers with StAR cDNA, GenBank accession number U17280

```
  1  CCAATGTCAAGGAGATCAAGGTC  23
     |||||||||||||||||||||||
572  CCAATGTCAAGGAGATCAAGGTC  594


  1  ACATTCATTACTCACGAGCTGGC  23
     |||||||||||||||||||||||
616  ACATTCATTACTCACGAGCTGGC  638
```

Fig. 6: SEQ ID NO.1:
amino acid sequence of human StAR

Length: 285 aa

```
  1   MLLATFKLCA  GSSYRHMRNM  KGLRQQAVMA  ISQELNRRAL  GGPTPSTWIN

 51   QVRRRSSLLG  SRLEETLYSD  QELAYLQQGE  EAMQKALGIL  SNQEGWKKES

101   QQDNGDKVMS  KVVPDVGKVF  RLEVVVDQPM  ERLYEELVER  MEAMGEWNPN

151   VKEIKVLQKI  GKDTFITHEL  AAEAAGNLVG  PRDFVSVRCA  KRRGSTCVLA

201   GMDTDFGNMP  EQKGVIRAEH  GPTCMVLHPL  AGSPSKTKLT  WLLSIDLKGW

251   LPKSIINQVL  SQTQVDFANH  LRKRLESHPA  SEARC
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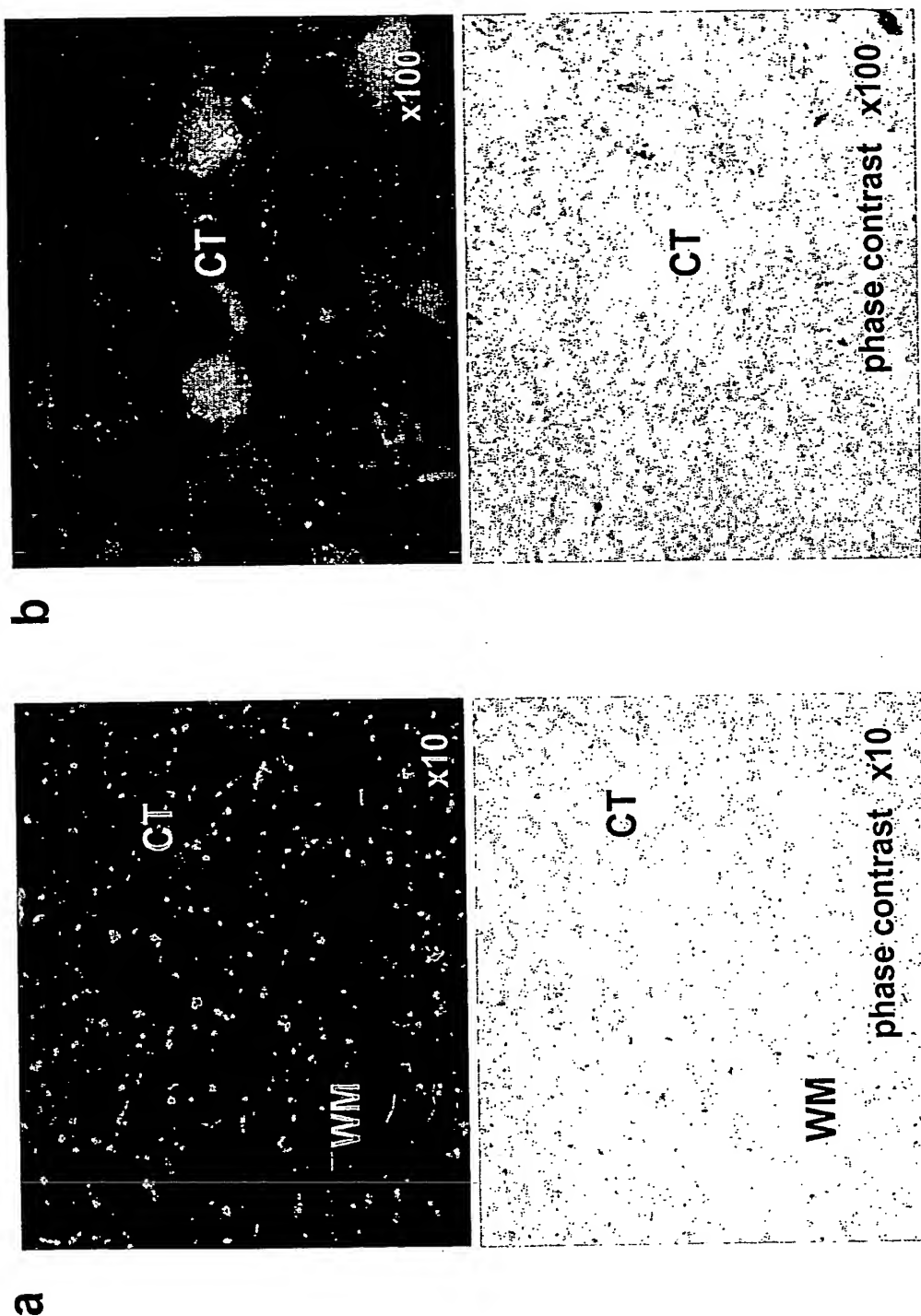Fig. 7: Images of the human cerebral cortex labeled with anti-StAR antibodies and with DAPI

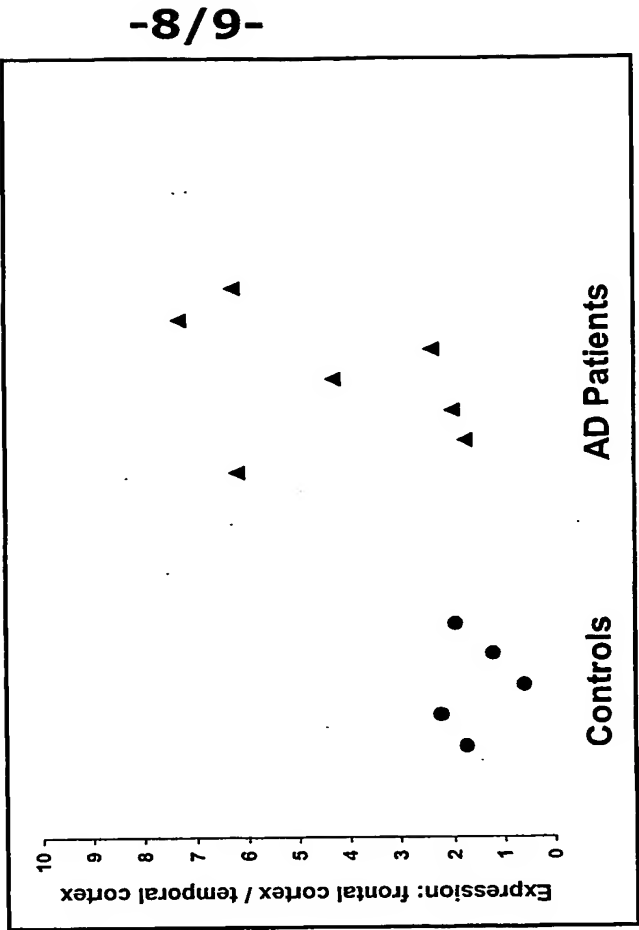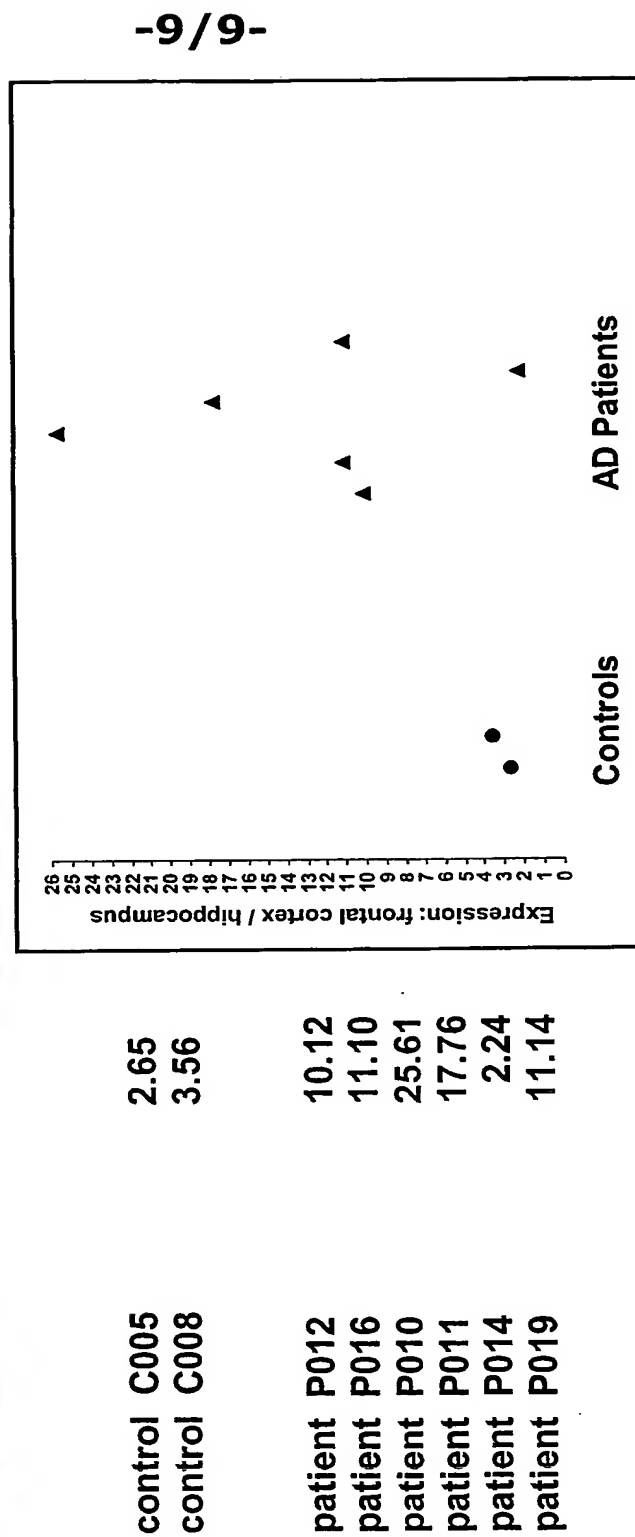Table 1 :

sample	Δ (fold) (frontal / temporal cortex)
control C011	1.74
control C012	2.25
control C014	0.59
control C005	1.22
control C008	1.96
patient P012	6.16
patient P016	1.72
patient P010	2.00
patient P011	4.28
patient P014	2.36
patient P017	7.30
patient P019	6.23

Expression: frontal cortex / temporal cortex

Controls　　　AD Patients

Table 2:

sample	Δ (fold) (frontal cortex / hippocampus)
control C005	2.65
control C008	3.56
patient P012	10.12
patient P016	11.10
patient P010	25.61
patient P011	17.76
patient P014	2.24
patient P019	11.14

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<120> Diagnostic and therapeutic use of steroidogenic acute
      regulatory protein for neurodegenerative diseases

<130> 031347wo ME/BM

<140>
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             20   25   30

Gln Glu Leu Asn Arg Arg Ala Leu Gly Gly Pro Thr Pro Ser Thr Trp
         35   40   45

Ile Asn Gln Val Arg Arg Arg Ser Ser Leu Leu Gly Ser Arg Leu Glu
     50   55   60

Glu Thr Leu Tyr Ser Asp Gln Glu Leu Ala Tyr Leu Gln Gln Gly Glu
 65   70   75   80

Glu Ala Met Gln Lys Ala Leu Gly Ile Leu Ser Asn Gln Glu Gly Trp
                 85   90   95

Lys Lys Glu Ser Gln Gln Asp Asn Gly Asp Lys Val Met Ser Lys Val
            100   105   110

Val Pro Asp Val Gly Lys Val Phe Arg Leu Glu Val Val Val Asp Gln
            115   120   125

Pro Met Glu Arg Leu Tyr Glu Glu Leu Val Glu Arg Met Glu Ala Met
        130   135   140

Gly Glu Trp Asn Pro Asn Val Lys Glu Ile Lys Val Leu Gln Lys Ile
145   150   155   160

Gly Lys Asp Thr Phe Ile Thr His Glu Leu Ala Ala Glu Ala Ala Gly
                165   170   175

Asn Leu Val Gly Pro Arg Asp Phe Val Ser Val Arg Cys Ala Lys Arg
            180   185   190

Arg Gly Ser Thr Cys Val Leu Ala Gly Met Asp Thr Asp Phe Gly Asn
        195   200   205

Met Pro Glu Gln Lys Gly Val Ile Arg Ala Glu His Gly Pro Thr Cys
    210   215   220

Met Val Leu His Pro Leu Ala Gly Ser Pro Ser Lys Thr Lys Leu Thr
225   230   235   240

Trp Leu Leu Ser Ile Asp Leu Lys Gly Trp Leu Pro Lys Ser Ile Ile
                    245   250   255

Asn Gln Val Leu Ser Gln Thr Gln Val Asp Phe Ala Asn His Leu Arg
                260   265   270

Lys Arg Leu Glu Ser His Pro Ala Ser Glu Ala Arg Cys
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(51) International Patent Classification[7]:   G01N 33/68, A61P 25/28, A01K 67/027, C12N 15/00, C07K 14/705

(21) International Application Number:
PCT/EP2003/005910

(22) International Filing Date: 5 June 2003 (05.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02012815.3   10 June 2002 (10.06.2002)   EP
60/386,721   10 June 2002 (10.06.2002)   US

(71) Applicant (for all designated States except US): EVOTEC NEUROSCIENCES GMBH [DE/DE]; Schnackenbur-gallee 114, 22525 Hamburg (DE).
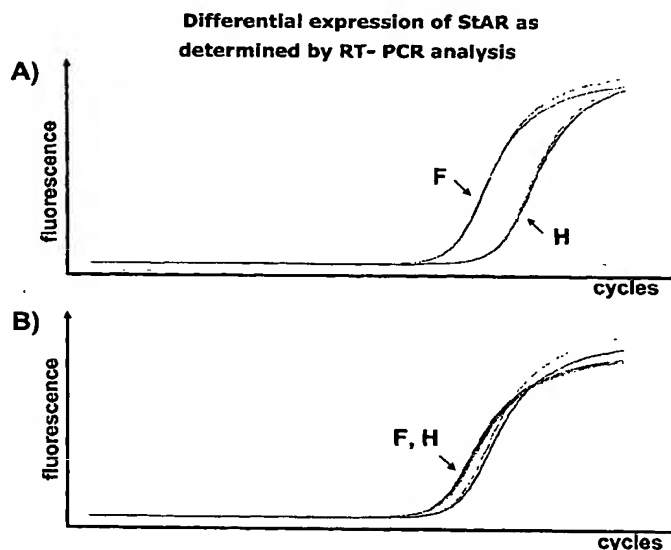
(72) Inventors; and
(75) Inventors/Applicants (for US only): HESTERKAMP, Thomas [DE/DE]; Nordhäuser Weg 18, 22455 Hamburg (DE). VON DER KAMMER, Heinz [DE/DE]; Verbindungsstr. 6d, 22607 Hamburg (DE). KRAPPA, Ralf [DE/DE]; Rostocker Str. 12, 25436 Tornesch (DE).

POHLNER, Johannes [DE/DE]; Quittenweg 11, 22175 Hamburg (DE).

(74) Agents: MEYERS, Hans-Wilhelm et al.; Patent Attorneys, von Kreisler Selting Werner, P.O. Box 10 22 41, 50462 Cologne (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

[Continued on next page]

(54) Title: DIAGNOSTIC AND THERAPEUTIC USE OF STEROIDOGENIC ACUTE REGULATORY PROTEIN FOR NEURODEGENERATIVE DISEASES

Differential expression of StAR as determined by RT-PCR analysis

(57) Abstract: The present invention discloses the differential expression of the steroidogenic acute regulatory protein (StAR) gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or for determining whether a subject is at increased risk of developing such a disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using the StAR gene and gene products. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

WO 2003/104811 A3